
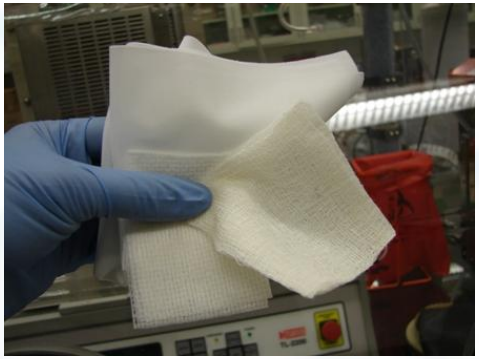


Enabling Forensics Investigations of Biothreat Incidents through Sampling Standards

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NIST
National Institute of Standards and Technology
U.S. Department of Commerce

MATERIAL MEASUREMENT LABORATORY

Phases of Response and Recovery to a Biological Incident

Response and Recovery*				
Crisis Management		**Consequence Management**		
		Remediation/Cleanup		
Notification	**First Response**	**Characterization**	**Decontamination**	**Clearance**
Receive information on biological incident Identification of suspect release sites **Notification of appropriate agencies**	**Initial threat assessment** **HAZMAT and emergency actions** **Start of Forensic investigation** Public health actions **Screening sampling** **Determination of agent type, concentration, and viability** Risk communication	**Characterization of biological agent** Characterization of affected site Site containment Continue risk communication **Characterization environmental sampling and analysis** Initial risk assessment Clearance goals	Decontamination strategy Remediation Action Plan Worker health and safety Site preparation Source reduction Waste disposal Decontamination of sites or items **Decontamination verification**	**Clearance environmental sampling and analysis** Clearance decision

Blue, NIST historical presence

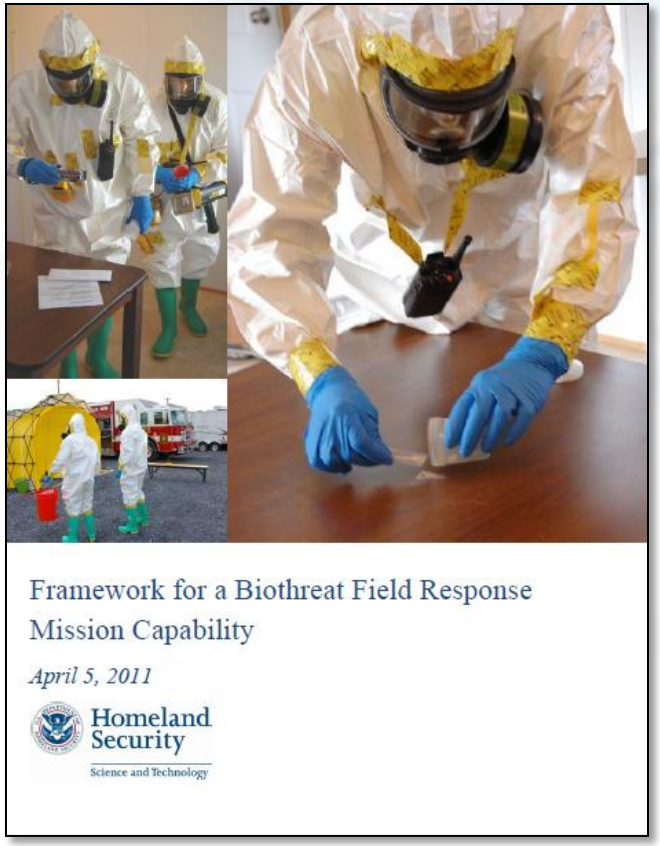
Red, Current NIST program expansion

Taken from **Planning Guidance for Recovery Following Biological Incidents**, May 2009, EPA/DHS

Framework for a Biothreat Field Response Mission Capability

Develop guidance to first responders for the biological assessment of suspicious powders

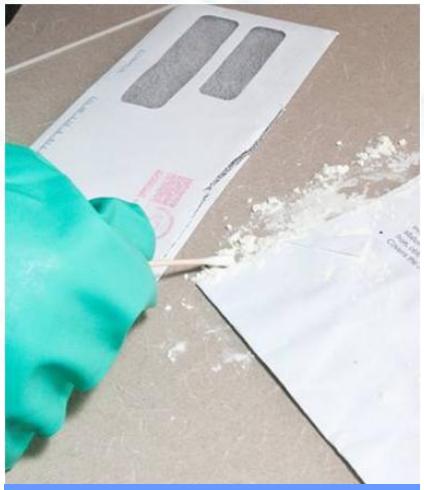
- Interagency effort involving DHS, CDC, FBI, and EPA
- Defines Critical Elements of a Mission Capability (a.k.a., an Actionable Assay – the Onion)
- Outlines the accomplishments and remaining gaps



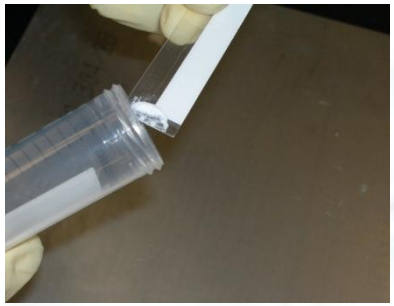
https://www.rkb.us/

Phase 1 of Response: Collection and Transport of Suspect Material to the Lab

ASTM E2458-10 Method B



Sample Collection



Packaged for Laboratory Analysis

ASTM E2458-10 Method A

Extraction

Assay Integration

Assay Integration and Communication of Results

4

ASTM E2458 Collection of Suspicious Powders

Method A – Bulk Sample Collection Method for Laboratory Analysis

- Method for collection of bulk of visible suspicious powder on nonporous surface
- Ensures sufficient sample is available to Laboratory Response Network (LRN) reference laboratory for confirmatory analysis

Method B – Swab Sample Collection for On-Site Analysis

- AFTER Method A applied, residual powder can be collected from surfaces
- Sample can be used for on-site biological assessments using biothreat field detection devices

ASTM E2770 Operational Guidance

Standard Guide - provides operational guidelines for initial response to a suspected biothreat agent

- Fundamentals for response planning to assure proper involvement, communication and coordination between key players in a jurisdiction
- Minimum training and PPE requirements for field personnel
- Guidance for risk assessment process to determine if visible powder should be deemed a biological threat
- Guidance for threat evaluation process in conjunction with law enforcement representatives (including FBI) for determination of threat credibility

Process Coordination
ASTM Standards E2770 and E2458

1

Initial sample screen, minimizing consumption

2

Communication of results to Law Enforcement and Public Health

3

Decision to collection with ASTM E2458

Initial Response Guidance and Collection Method

Designation: E2458 – 10

Standard Practices for Bulk Sample Collection and Swab Sample Collection of Visible Powders Suspected of Being Biothreat Agents from Nonporous Surfaces[1]

This standard is issued under the fixed designation E2458; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 These practices address collection of visible powders that are suspected biothreat agents from solid nonporous surfaces using a bulk collection method, using a dry swab and laminated card, followed by a swab sampling method using a sterile moistened swab. Bulk powder samples are collected and packaged in a manner that permits the maximum amount of the sample to be safely transported to a reference laboratory within the Centers for Disease Control and Prevention (CDC) national Laboratory Response Network (LRN)[2] for confirmatory identification and safe storage. If the source of the powder is a letter or small package, that item is also packaged in a manner that permits it to be safely transported to an LRN reference laboratory. A sterile moistened swab may be used to collect residual powder and may be used to conduct on-site biological assessments for the purpose of testing for biothreat agents.

1.2 These practices are performed in coordination with the Federal Bureau of Investigation (FBI) as part of a risk assessment including hazard assessment and threat evaluation as recommended and clarified in Guide E2770. The decision to implement these practices and collect a public safety sample will be made by members of the response community of the jurisdiction assuming responsibility through coordination with the FBI and the receiving LRN reference laboratory.

1.3 Sample Collection Method A covers the bulk collection and packaging of suspicious visible powders that are suspected biothreat agents from solid nonporous surfaces. All samples suspected to be biothreat agents on nonporous surfaces should be collected according to Sample Collection Method A and sent to a LRN reference laboratory for confirmatory testing.

1.4 Sample Collection Method B covers swab sampling of residual suspicious powders that are suspected biothreat agents from solid nonporous surfaces. Swab samples can be used for on-site biological assessment; however results from on-site biological assessments are not definitive; confirmatory testing by the LRN reference laboratory is necessary to make public health decisions.

1.5 These practices incorporate reference guidance for packaging and transport of suspicious visible powders to comply with all appropriate federal regulations regarding biosafety and biosecurity.

1.6 These practices should only be used to collect visible samples that are suspected biothreat agents and have been field screened according to reference guidance for explosive hazard, radiological hazard, and other acute chemical hazards.

1.7 The bulk sample collection practice and the swab sampling practice are recommended for collecting amassed or dispersed powder samples from all nonporous surfaces on which the suspicious powder sample is clearly visible.

1.8 These practices are not recommended for samples on porous materials such as upholstery, carpeting, air filters, or ceiling tiles.

1.9 These practices are recommended for collecting visible powders where the bulk of the powder sample is amassed or dispersed over a limited area (optimally, area should be less than 20 by 20 cm (approximately 8 by 8 in.) or 400 cm^2 (approximately 64 $in.^2$).

1.10 These practices are to be performed by personnel who are adequately trained to work with hazardous materials in the hot zone (see NFPA 472, or OSHA 1910.120). Personnel performing collection or screening under these practices shall be adequately trained in the use of sampling equipment, materials, and procedures. This includes personnel performing the prior initial chemical and radiological screening. Personnel should use the appropriate level of personal protective equipment (PPE) to mitigate hazards during collection and screening. Personnel performing collection or screening under these practices shall be aware of evidence preservation and sampling procedures (NFPA 472 section 6.5).

[1] These practices are under the jurisdiction of ASTM Committee E54 on Homeland Security Applications and are the direct responsibility of Subcommittee E54.01 on CBRNE Sensors and Detectors.

Current edition approved Oct. 1, 2010. Published October 2010. Originally approved in 2006. Last previous edition approved in 2006 as E2458 – 06. DOI: 10.1520/E2458-10.

[2] The CDC Laboratory Response Network is the network responsible for handling clinical specimens and environmental samples containing suspected biothreat agents.

Designation: E2770 – 10

Standard Guide for Operational Guidelines for Initial Response to a Suspected Biothreat Agent[1]

This standard is issued under the fixed designation E2770; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

INTRODUCTION

A biothreat is a serious matter that affects public health, public safety, the economy and the general confidence of the people. The National Strategy for Homeland Security and in its National Response Framework focuses homeland security efforts on preventing and disrupting terrorist attacks, protecting the American people, our critical infrastructure and key resources; responding to and recovering from incidents that do occur while continuing to strengthen the foundation of our Nation. As laid out by the National Response Framework, a coordinated and synchronous response to suspected acts of bio-terrorism requires advance planning, including the equipping and training of emergency responders prior to an incident. The goal of this standard guide is to support national standards for responding to and collecting suspected biothreat agents with guidance centered on coordination among representatives of emergency response teams, including hazardous materials response teams, law enforcement, public health, including the Centers for Disease Control and Prevention (CDC) national Laboratory Response Network (LRN), and the Federal Bureau of Investigation (FBI). This standard guide provides uniform guidance that covers all of the following components: response planning, responder training, competency evaluation, proficiency testing, concept of operations, hazard assessment, threat evaluation, sample collection, field screening, risk communication and documentation for responding to visible powders suspected of being biothreat agents.

1. Scope

1.1 This guide provides considerations for decision-makers when responding to incidents that may involve biothreats. It provides information and guidance for inclusion in response planning, on activities to conduct during an initial response to an incident involving suspected biothreat agents.

1.2 This guide delineates fundamental requirements for developing a biothreat sampling and screening capability within a jurisdiction, practice, or operational area to assure proper involvement, communication, and coordination of all relevant agencies.

1.3 This guide applies to emergency response agencies that have a role in the initial response to a biothreat incident. It is designed for emergency response services such as law enforcement, fire departments, hazardous materials, public health, and emergency management.

1.4 This guide assumes implementation begins well before the recognition of a suspected biothreat event and ends when emergency response actions cease or the response is assumed by federal response teams.

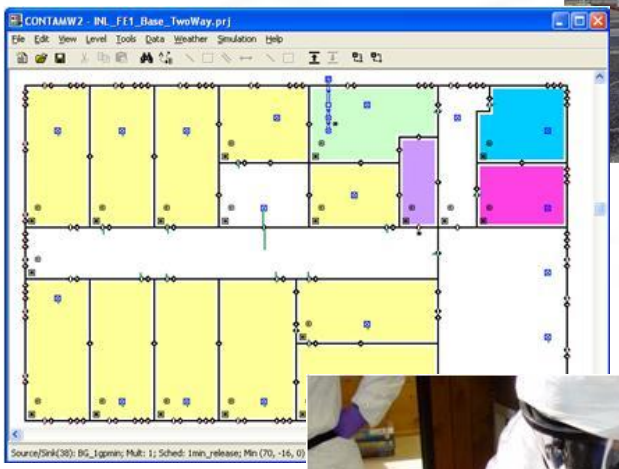
1.5 This guide utilizes risk-based response architecture and the guidance as described in the National Response Framework and is intended to be coupled with the authority having jurisdiction's (AHJs) understanding of local vulnerabilities and capabilities when developing its plans and guidance documents on response to incidents involving a suspected biothreat.

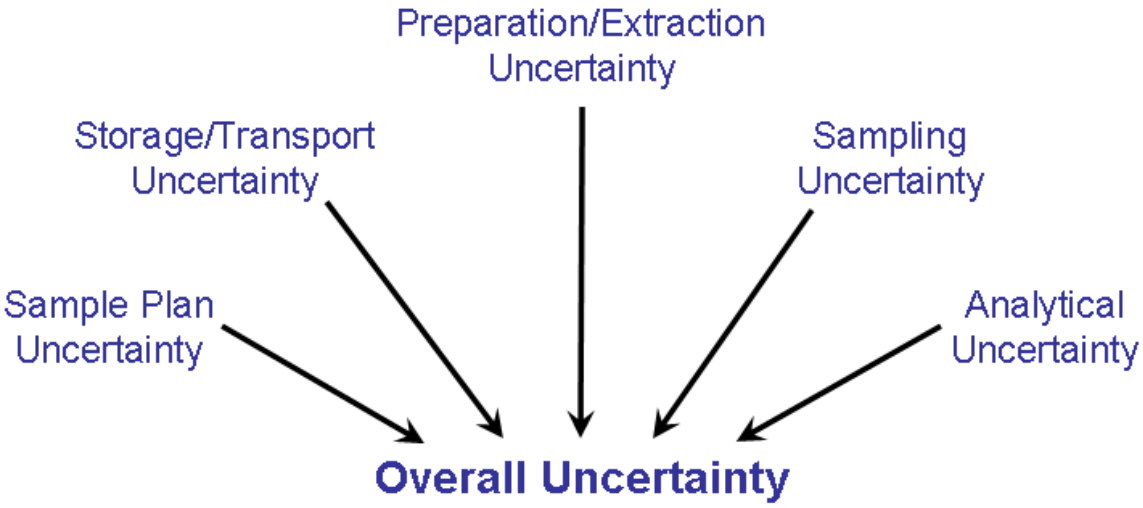
1.6 This guide is compliant with the National Incident Management System (NIMS) and uses Incident Command System (ICS) common terminology. Full compliance with NIMS is recognized as an essential part of emergency response planning. In developing this standard, every effort was made to ensure that all communications between organizational elements during an incident are presented in plain language

[1] This practice is under the jurisdiction of ASTM Committee E54 on Homeland Security Applications and is the direct responsibility of Subcommittee E54.01 on CBRNE Sensors and Detectors.

Current edition approved . Published XXXX 200X. DOI: 10.1520/E2770-10.

Phase 2 of Response: Characterize Sample Collection Performance

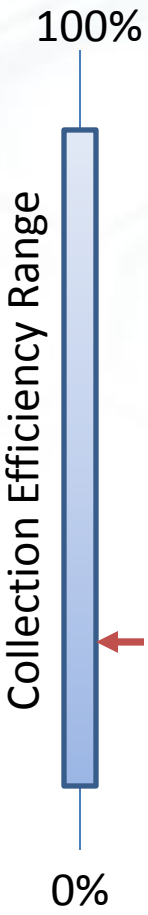




9

Current State-of-the-Art

High Degree of Variability in Sample Collection Data



- Deposition method: liquid, aerosolized (Ref method = 95% ETOH)
- Wetting agents: Water, PBS, +/- Surfactant
- Controlled Substrata: nonporous, carpets, porous
- Collection Method: wipes, swabs, vacuums
- Collection Material: rayon/polyester, rayon, cotton
- Processing Method: sonication, vortexing, stomacher
- Reporting: +/- Growth, qPCR, reference coupons

CDC Validation data, 2009

In 90+ papers dated 1964 to 2012

Collection Efficiencies Range from 7 to 87%

Challenges to Collection Performance

Microbial Sample

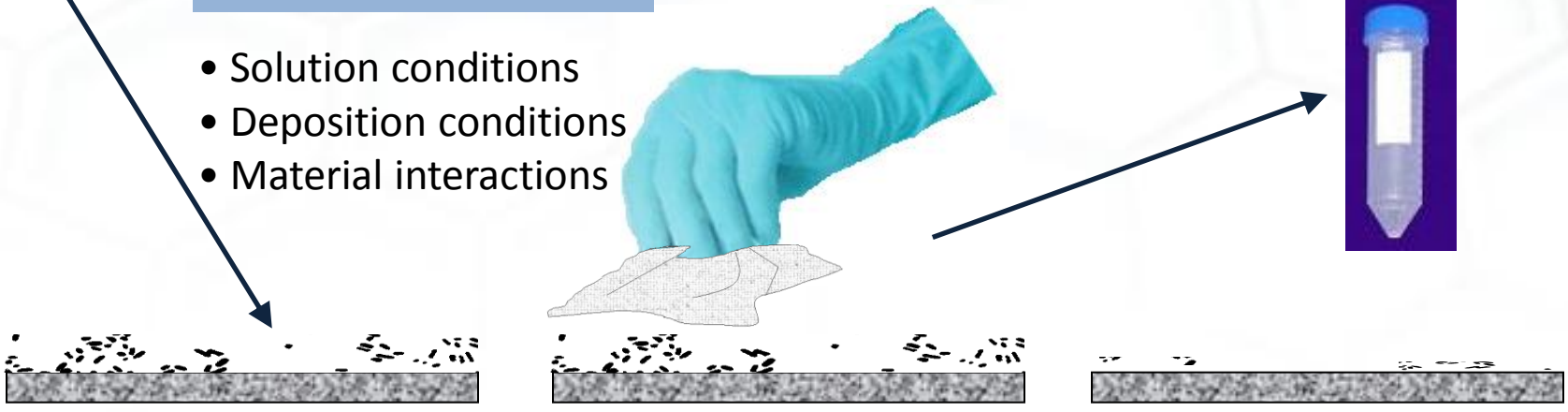
- Re-aerosolization
- Suspension stability
- Viability
- Quantity

Integration with Detection Technology

- Optimization of removal from wipe
- Interference with detection technologies
- Post-decon impacts on wipe extraction

Deposition method optimization

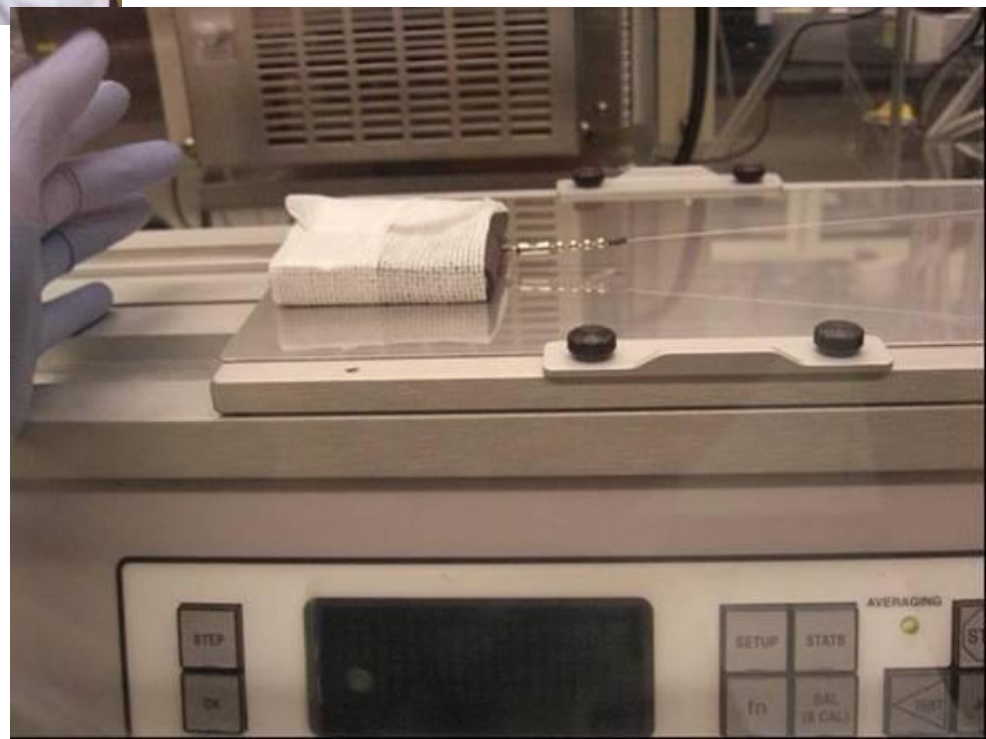
- Solution conditions
- Deposition conditions
- Material interactions



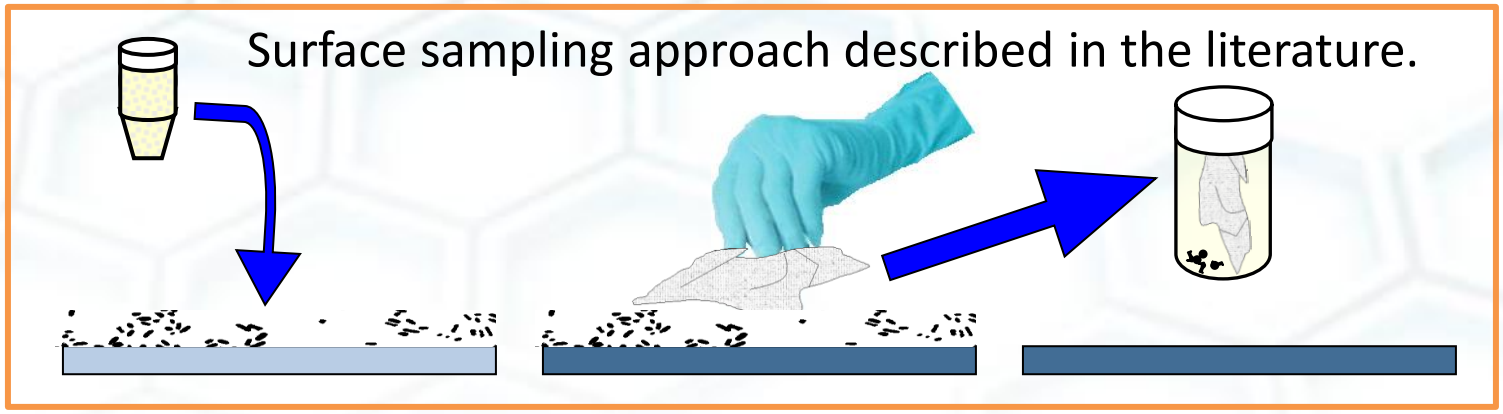
Optimization of Sampling Method

- Environmental Conditions
- Sampling pressure and velocity
- Mass balance on material for loss evaluation
- Wipe and substratum material interactions
- Post-decon impacts on wipe efficiency

Controlled Pressure and Environmental Conditions



Study Approach



Study 2

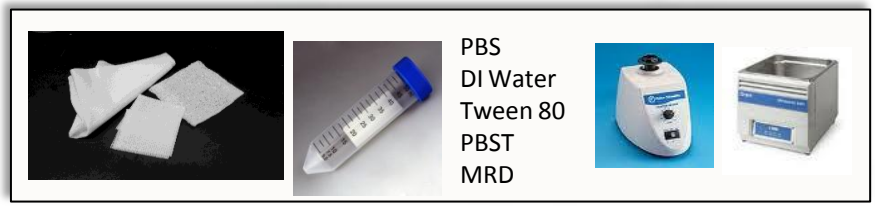
Collection step

Surface: Glass and Stainless steel
Relative humidity: 45% and 75%
Wetting agents: PBS, PBST, Tween 80 and DI water
Wipe materials: Polyester, cotton and polyester-rayon

Study 1

Extraction step

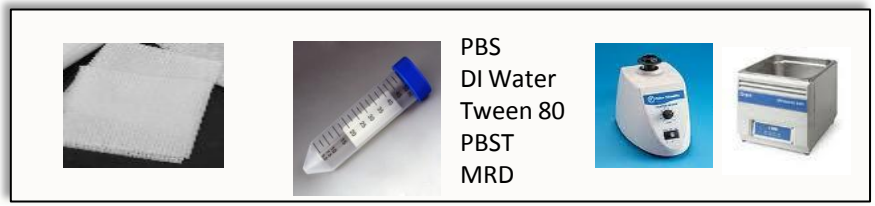
B. anthracis Sterne spores



Study 3

Extraction step

B. cereus, *E. coli* and *B. thailandensis*



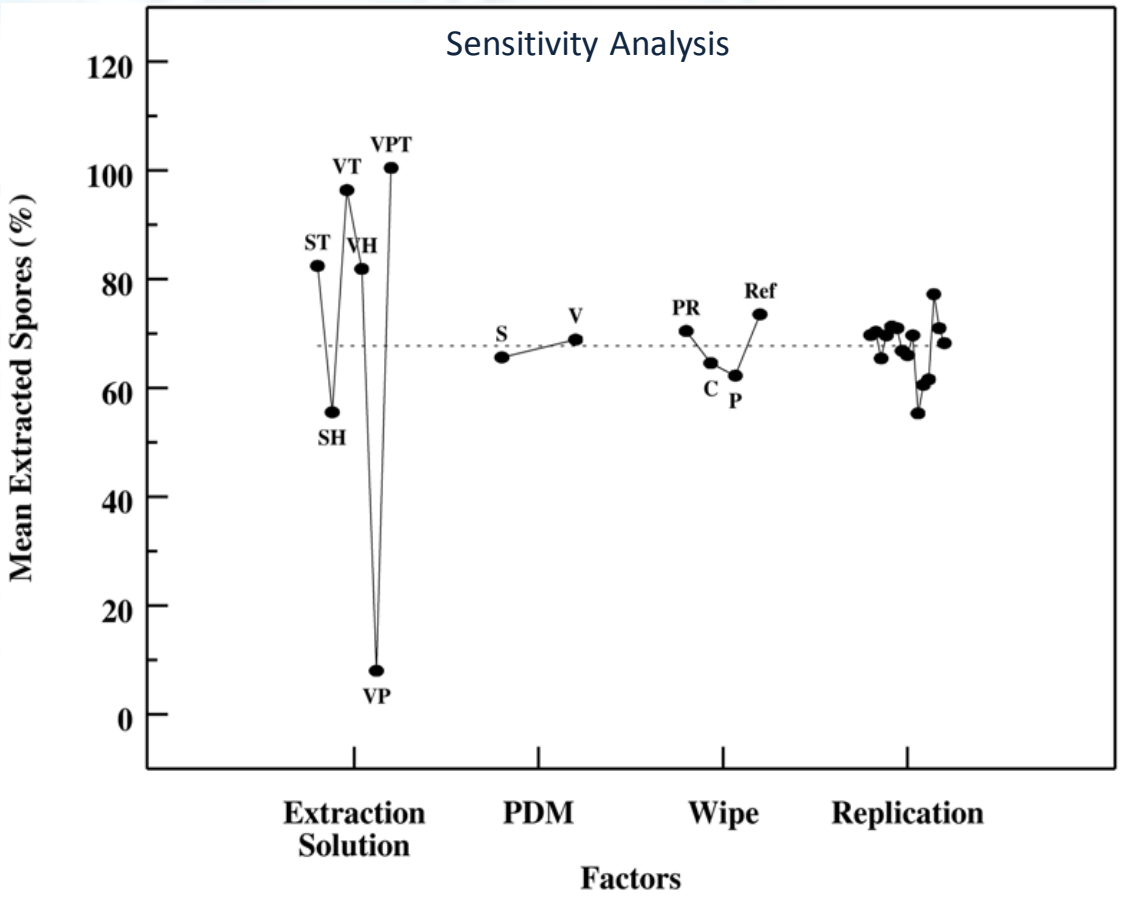
Study 1 – *B. anthracis* spores Processing and Extraction Performance

Method		Extraction Recovery % (SD)							
PDM	Solution	Poyester-rayon		Cotton		Polyester		Control	
Sonicate	H_2O	65.6	(14.2)	51.5	(14.7)	39.8	(16.9)	**65.5**	**(16.9)**
Sonicate	H_2O T80	89.1	(11.2)	77.2	(14.3)	74.9	(9.3)	**88.5**	**(15.1)**
Vortex	H_2O	87.1	(15.3)	68.3	(13.2)	83.3	(24.4)	**88.9**	**(27.2)**
Vortex	H_2O T80	90.5	(17.9)	96.4	(13.0)	102	(14.1)	**96.6**	**(15.4)**
Vortex	PBS	8.7	(3.6)	9.8	(3.3)	3.1	(2.2)	**10.4**	**(6.1)**
Vortex	PBS T80	99.0	(12.9)	101	(9.8)	91.9	(23.5)	**110**	**(12.2)**

PDM, Physical Dissociation Method
PBS, Phosphate Buffered Saline
T80, 0.04% Tween 80
~$2x10^4$ spores/wipe

$4^1 6^1$ replicated full factorial design, 24 combinations, each combination was replicated for n =264 as the total observations.

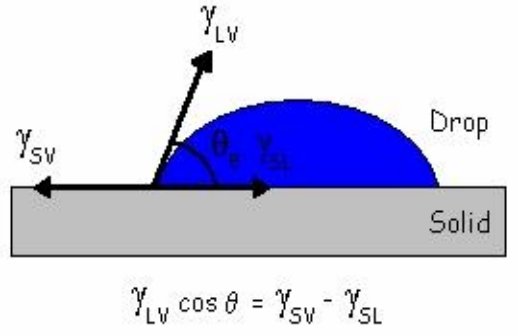
Study 1 – *B. anthracis* spores extraction

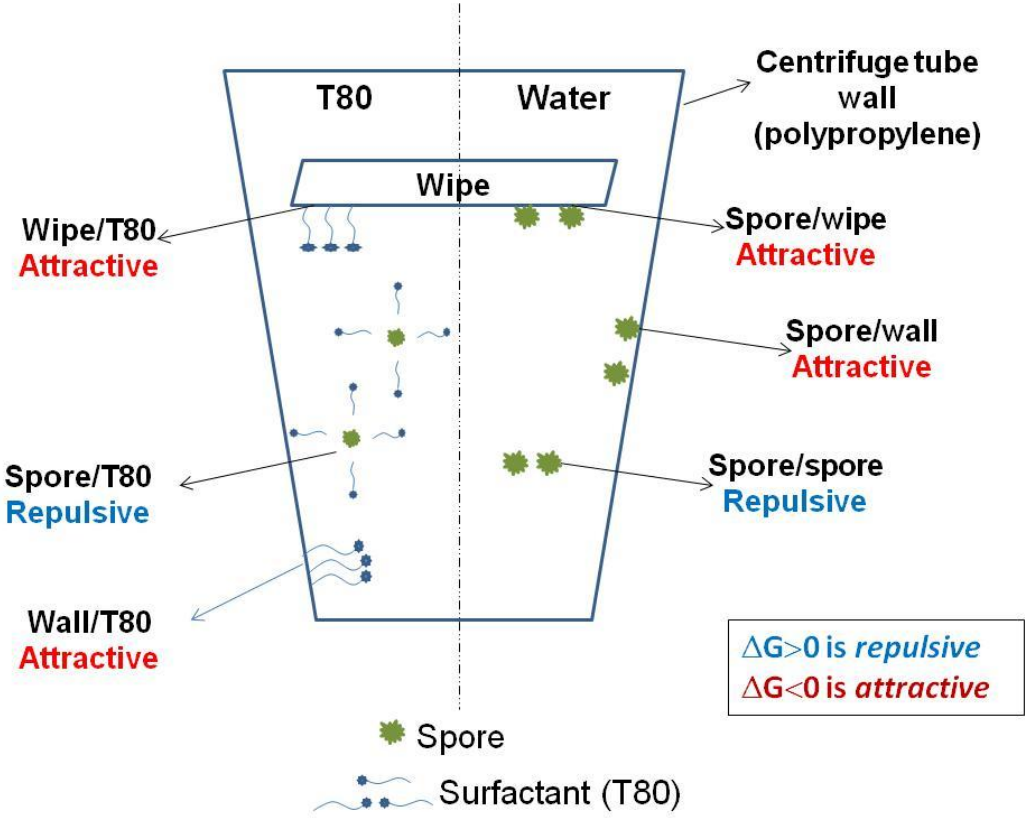


Extraction solution was the most important factor affecting recovery of *B. anthracis* due to interactions with centrifuge tubes explained by interfacial energy.

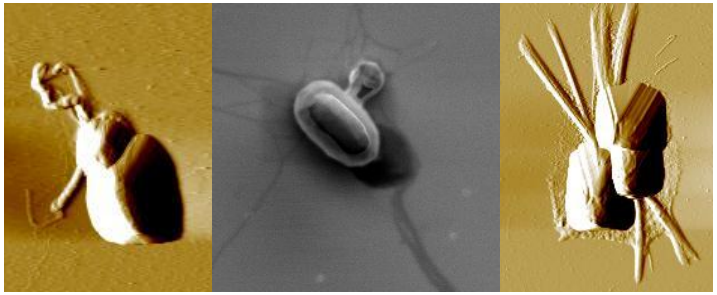
Da Silva SM, Filliben J J and Morrow JB., 2011, *Appl. Environ. Microbiol.*, 77(7), 2374-80.

Extraction and Recovery Performance: Interfacial Energy Impacts





Solutions with **surfactant** dramatically increased recoveries due to the interaction between the surfactant and the centrifuge tube wall preventing spore **adhesion**.

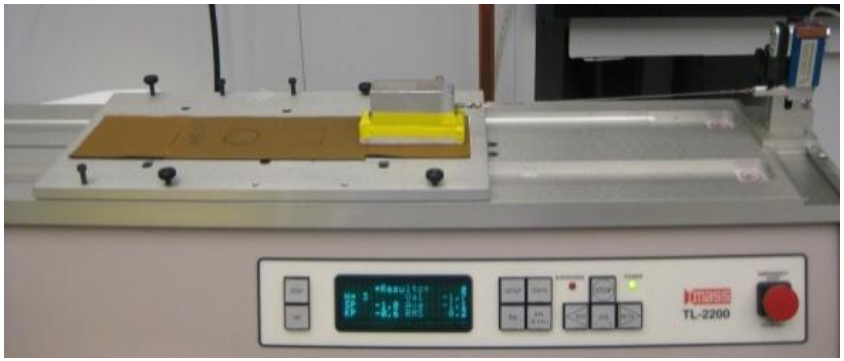


Study 2 – *B. anthracis* spores collection

Surface (Factor 1)	Wetting agent (Factor 2)	Relative Humidity (Factor 3)	Wipe (Factor 4)
Glass	PBS	45%	Polyester
Stainless steel	PBS + 0.04% Tween 80 (PBST)	75%	Cotton
	Sterile water		Polyester-rayon
	0.04% Tween 80		

~200 spores deposited per 1.2 cm^2 surface

Full factorial design (4x3x2x2) , 48 runs with additional selected runs to provide replication



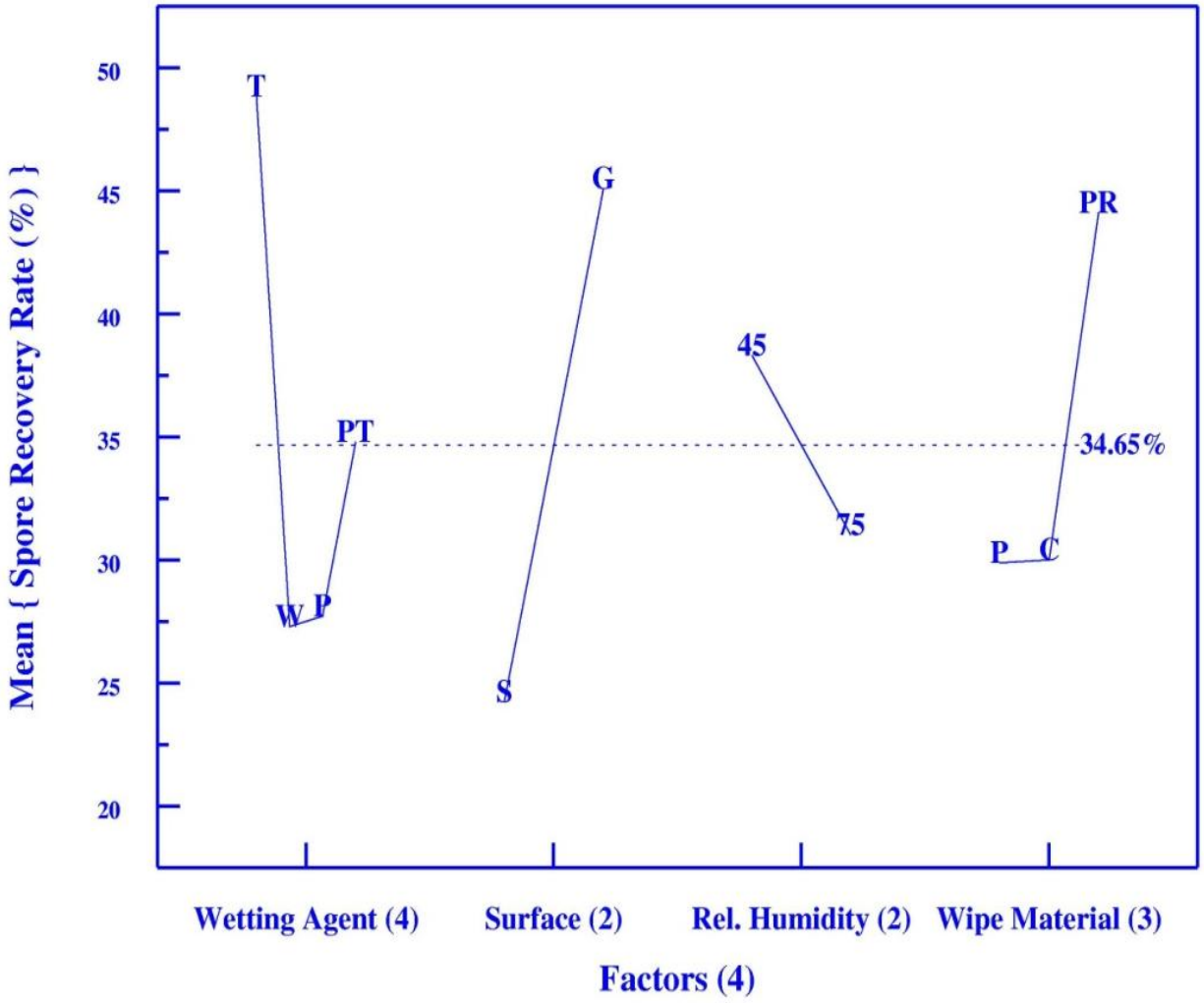
Slip/Peel tester

Stainless steel

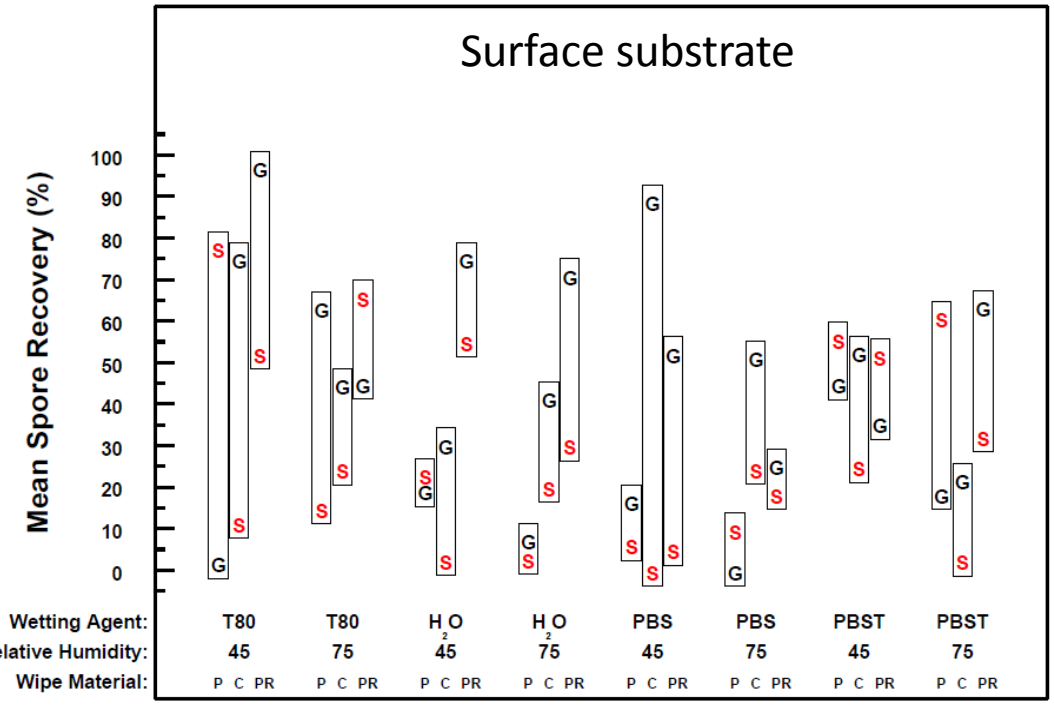
glass

Factors Impacting Recovery Performance

- **Surface:** Glass and steel
- **Relative humidity:** 45% and 75%
- **Wetting agent:** PBS, PBST, Tween 80 and DI water
- **Wipe:** Polyester, cotton and polyester-rayon



Study 2 – *B. anthracis* spores collection



Wetting Agent (4) x Relative Humidity (2) x Wipe Material (3) = 24 Combinations

Roughness (Ra)
Glass = 0.0018 µm
Steel = 0.1628 µm

Glass recovery was higher for 17 out of 24 combinations (p=0.0113)

Wetting agent: T80, H_2O, PBS, PBST ($p > 0.05$)
Relative humidity: 45% and 75% ($p > 0.05$)
Wipe: polyester, cotton and polyester-rayon ($p > 0.05$)

Rank analysis: T80, Glass, 45%RH and Polyester-rayon provided the best result

- Produced guidance for the first responder community for collection of suspected biothreat agents
  - ASTM E2770 and E2458
  - Joint publication with NIOSH on sampling from porous and carpeted surfaces, NIST TN 1776
  - Field Operational Exercises
  - Collection App http://webpub.nist.gov/suspiciouspowders
- Published sources of uncertainty in sample collection procedures to enhance confidence in the current technologies and protocols (recovery efficiencies for *B. anthracis spores,* vegetative *B. cereus, E. coli, Burkholderia thailandensis)*

Da Silva et. al. JAM, 2012, accepted

Downey, et. al. AEM, 2012, 78(16):5872-81

Da Silva et. al. AEM, 2011, 77(7), 2374-80

Areas of potential impact and future measurement challenges

Monday 10 January 2011

The Telegraph

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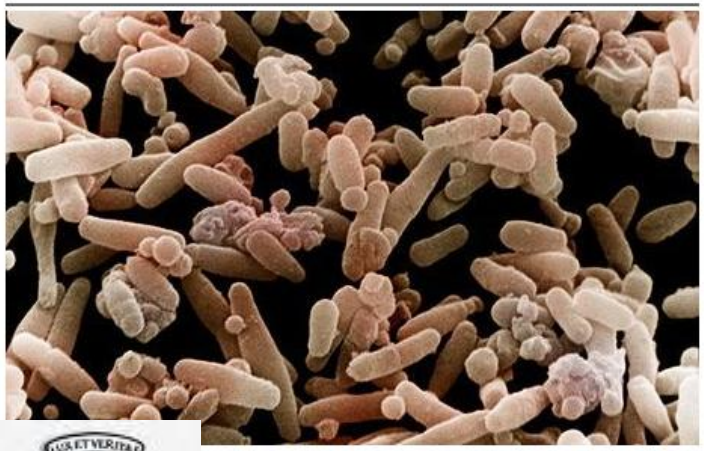
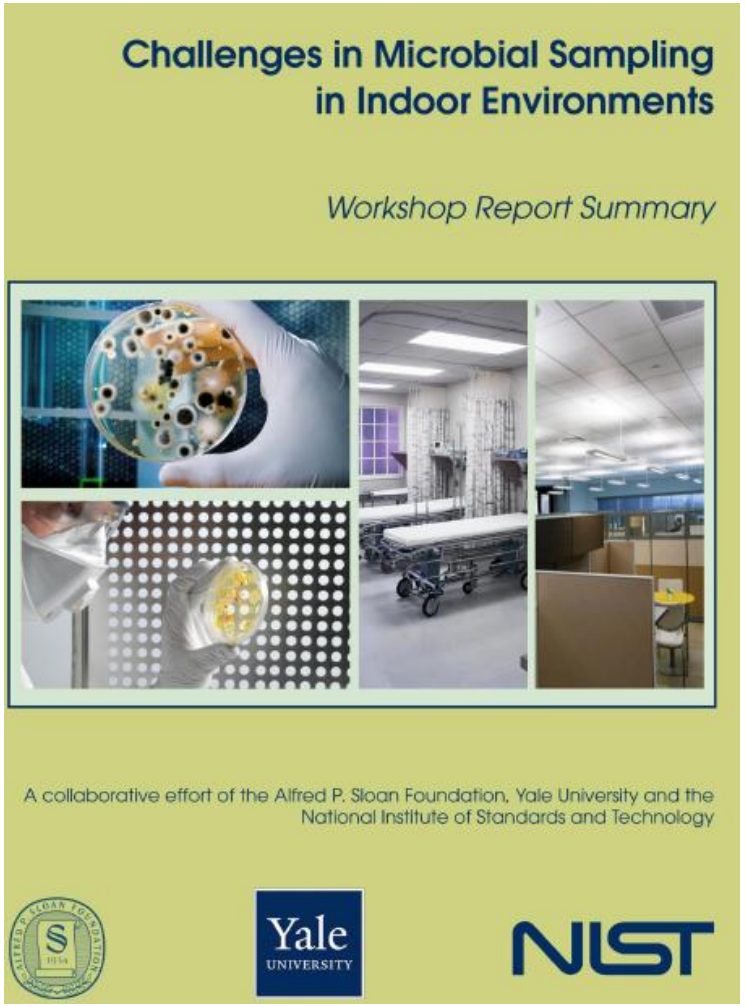
Science

Human Microbiome Project: a map of every bacterium in the body

The Human Microbiome Project is analysing our microbes to advance medicine. By Michael Day .



Our intestinal tract is home to 10 trillion organisms. Photo: SCIENCE



Sampling the Indoor Environment Workshop, February 14-15, 2011

YALE

ALFRED P. SLOAN FOUNDATION

Acknowledgements

Sandra M. Da Silva, Autumn S. Downey,
Nate Olson, Lindsay Vang
Biosystems and Biomaterials Division

Greg Gillen, Jennifer Verkouteren
Surface and Microanalysis Division

Jim Filliben
Statistical Engineering Division

Bert Coursey
Office of Special Programs

All the members of the response community that have volunteered their thoughts, time and expertise to the development and creation of the standards

APHL
ASSOCIATION OF PUBLIC HEALTH LABORATORIES

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

DEPARTMENT OF JUSTICE · FEDERAL BUREAU OF INVESTIGATION · FIDELITY · BRAVERY · INTEGRITY

U.S. DEPARTMENT OF HOMELAND SECURITY

UNITED STATES · ENVIRONMENTAL PROTECTION AGENCY

INTERNATIONAL FIRE CHIEFS ASSOCIATION

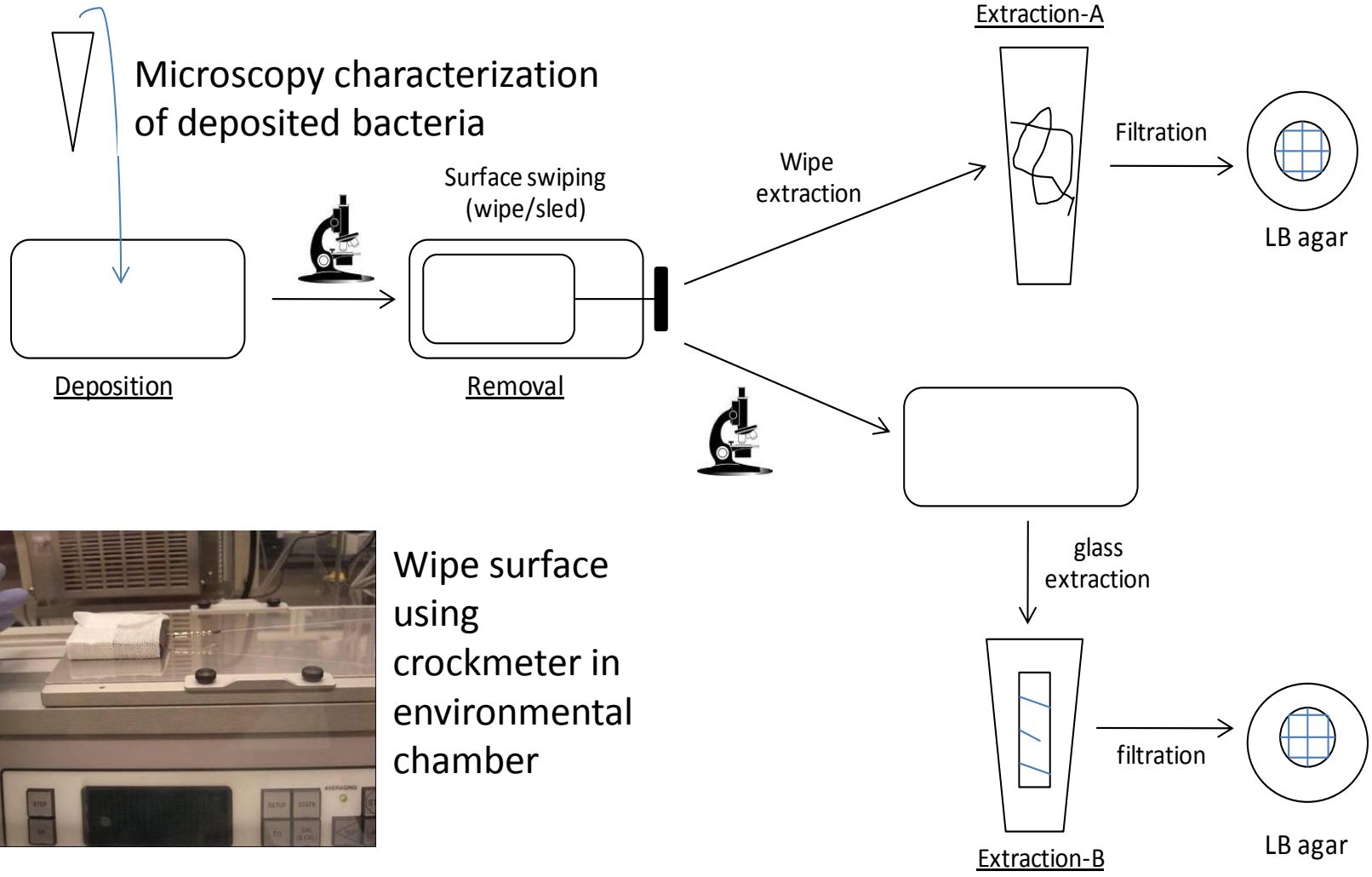
"Out of Many, One" · Local, State, and Federal Standardization and Interoperability

Acknowledgment

The Department of Homeland Security (DHS) Science and Technology Directorate sponsored the production of this material under Interagency Agreement HSHQDC-09-X-00457 with the National Institute of Standards and Technology (NIST).

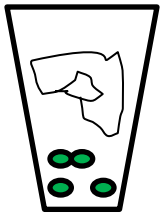
Well characterized aqueous bacterial suspension

Enhanced extraction efficiency by solution chemistry manipulation



Wipe surface using crockmeter in environmental chamber

NIST Analytical Approach



Interactions

Calculated interfacial energy, ΔG, for surface 1 and surface 2 immersed in water (3).

	$\Delta G_{131}^{a,b}$ (mJ/m^2)
BA spores (1)[c], BA spores (1)[c]	31.68
BA spores(1)[d], BA spores (1)[d]	33.76
	ΔG_{132} (mJ/m^2)
BA spores(1)[c], polypropylene(2)	-9.25
BA spores(1)[c], Polyester(2)	4.34
BA spores(1)[c], Cotton(2)	-8.49
BA spores(1)[c], Polyester-rayon(2)	-16.87

	ΔG_{132} for Tween 80 surface films[e]	
	Tween 80 head group(2)	**Tween 80 tail group(2)**
BA spores[c](1)	21.5	6.99
Polyester(1)	-17.98	-53.99
Cotton(1)	4.17	-7.15
Polyester-rayon(1)	-44.91	-71.05
Polypropylene(1)	-36.6	-75.28

[a]Interfacial energy subscripts are denoted. [b] $G<0$ is attractive, $\Delta G>0$ is repulsive.

[c] BA spore surface tension measured in deionized water (Table 2). [d] BA spore surface tension measured in PBS buffer .

[e]Interfacial energy calculations were performed for surfaces with Tween 80 moieties exposed at the interface